UNIVERSITI PUTRA MALAYSIA

CHEMICAL CONSTITUENTS, BIOACTIVITY AND HPLC PROFILING OF MICROWAVE-ASSISTED AND NORMAL EXTRACTION OF *Murraya koenigii*

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By

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June 2008

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Faculty: **Faculty of Science**

Murraya koenigii (L.) Spreng is an aromatic plant, which is normally used as natural flavoring in curries and sources, and commonly found in India and Peninsular Asia. Locally, *Murraya koenigii* is known as curry leaf tree and one of the richest source of carbazole alkaloids. It has been reported that carbazole alkaloids possess various biological activities such as anti-tumor, anti-oxidative, anti-mutagenic and anti-inflammatory activities. In this work, six carbazole alkaloids: mahanimbine (**33**), girinimbine (**17**), murrayanine (**31**), murrayafoline-A (**62**), murrayacine (**39**) and 3-methylcarbazole (**63**), one long chain ester (**61**) together with *β*-sitosterol (**24**) were isolated from leaves, stem barks, and roots of *Murraya koenigii* by using normal extraction (NE) and microwave-assisted extraction (MAE). The structures of these compounds were established by spectroscopic method and by comparison with the previous reported works.

The essential oil of the fresh curry leaves was obtained using conventional hydrodistillation (CHD) and microwave-assisted hydrodistillation (MAHD), and were analyzed by gas chromatography and GC-MS techniques. Most of the components of the oils obtained from both techniques of distillation were rather similar but with different variations of quantities. In the essential oil obtained from conventional hydrodistillaion (CHD), the major compound was α-farnesene (18.74%), whereas the major constituent of oils from microwave-assisted hydrodistillation (MAHD) was 4,11,11-trimethyl-8-methylenebicycloundec-4-ene (29.67%).

All crude extracts and carbazole alkaloids isolated from *Murraya koenigii* were screened their cytotoxic activities towards human T-promyelocytic leukemic cell lines (HL-60), human colon cancer cells (HT-29), human breast cancer cells (MCF-7) and human cervical carcinoma cancer cells (HeLa) and all isolated compounds were strongly active with IC_{50} values gave less than 20 μg/ml. In the larvicidal activity, the crude extracts and isolated compounds were tested against the larvae of *Aedes aegypti*. All crude extracts and isolated compounsds showed very strong activity against *Aedes aegypti* with LC_{50} values of between 0.68 ppm to 55.03 ppm. In the antimicrobial screening, only crude chloroform extract of roots of *Murraya koenigii* showed low activity against *Bacillus subtilis,* while the antifungal test showed that the crude chloroform extract of roots and murrayafoline-A (**62**) showed low activity against the *Candida albicans.*

The bioactivity tests carried out in this research which include antimicrobial activity of some pathogenic microbes, cytotoxicity tests against some cancer cell lines (HL-60, MCF-7, HT-29 and HeLa) and larvicidal activity properties against *Aedes aegypti* were

never been reported previously. In addition, the microwave-assisted extraction of the plant and the development of the profiling of the extracts based on using HPLC-UV technique were never been investigated before.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan ijazah Master Sains

PEMENCILAN SEBATIAN KIMIA, AKTIVITI-AKTIVITI BIOLOGI DAN PEMPROFILAN KROMATOGRAFI CECAIR PRESTASI TINGGI BAGI PENGEKSTRAKAN GELOMBANG MIKRO DAN PENGEKSTRAKAN NORMAL DARIPADA *Murraya koenigii*

Oleh

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Murraya koenigii (L.) Spreng adalah tumbuhan aromatik, yang biasanya di gunakan sebagai perisa semulajadi dalam masakan kari dan sebagainya, selalunya di jumpai di India dan Peninsular Asia. *Murraya koenigii* juga dikenali sebagai pokok daun kari dan merupakan salah satu sumber yang kaya dengan karbazol alkaloid. Karbazol alkaloid pernah dinyatakan bagi pelbagai aktiviti biologi antaranya ialah anti-kanser, anti-penuaan, anti-mutagenik dan anti-radang. Dalam kajian ini, enam karbazol alkaloid: mahanimbin (**33**), girinimbin (**17**), murrayanin (**31**), murrayafolin-A (**62**), murrayacin (**39**) dan 3-metilkarbazol (**63**), ester rantai panjang (**61**) bersama *β*-sitosterol (**24**) telah dipencilkan daripada bahagian daun, kulit batang dan akar *Murraya koenigii* dengan menggunakan kaedah pengekstrakan biasa (rendaman) (NE) dan kaedah pengekstrakan

menggunakan gelombang mikro (MAE). Kesemua sebatian telah dicirikan berdasarkan data spektroskopi dan perbandingan dengan data kajian sebelum ini.

Minyak pati daripada daun kari yang segar didapati dengan menggunakan kaedah penyulingan hidro (CHD) dan penyulingan hidro dengan gelombang mikro (MAHD), minyak pati di analisis menggunakan kaedah spektroskopi GC-MS. Kebanyakan komponen dalam minyak pati yang terhasil daripada kedua-dua teknik penyulingan tersebut adalah agak sama tetapi berbeza dari segi kuantiti. Sebatian utama minyak pati yang terhasil daripada penyulingan hidro (CHD) adalah α-farnesena (18.74%), dan bagi penyulingan hidro dengan gelombang mikro (MAHD) pula adalah 4,11,11-trimetil-8-metilenabisikloundek-4-ena (29.67%).

Kesemua ekstrak dan sebatian tulen daripada *Murraya koenigii* juga telah disaring dengan aktiviti sitotoksik terhadap sel kanser leukemia manusia (HL-60), sel kanser kolon manusia (HT-29), sel kanser payudara manusia (MCF-7) dan sel kanser servik manusia (HeLa), dan menunjukkan aktiviti yang tinggi dengan nilai IC_{50} adalah kurang daripada 20 μg/mL. Dalam ujian aktiviti larva, kesemua ekstrak dan sebatian tulen telah diuji terhadap larva nyamuk Aedes (*Aedes aegypti)*. Kesemua ekstrak dan sebatian tulen mempamerkan aktiviti yang tinggi terhadap larva *Aedes aegypti* dengan nilai LC_{50} adalah di antara 0.68 ppm hingga 55.03 ppm. Bagi penyaringan antimikrobial pula, hanya ekstrak klorofom daripada akar *Murraya koenigii* menunjukkan aktiviti yang rendah terhadap *Bacillus subtilis,* sementara dalam ujian antifungal ekstrak klorofom daripada akar dan murrayafoline-A aktiviti juga menunjukkan aktiviti yang rendah terhadap *Candida albicans.*

Penyelidikan ini diteruskan dengan mengkaji aktiviti biologi bagi aktiviti anti-mirob terhadap beberapa mikrob, ujian anti-sitotoksik melawan sel kanser (HL-60, MCF-7, HT-29 and HeLa) dan aktiviti larvicidal melawan *Aedes aegypti* kerana telah dikaji tiada laporan terdahulu mengenainya. Sebagai penambahan, teknik pengekstrakan gelombang mikro telah dibangunkan untuk pemprofilan bagi ekstrak menggunakan teknik HPLC-UV juga telah dikaji tiada penyelidikan mengenainya.

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I certify that an Examination Committee has met on 21st October 2008 to conduct the final examination of Noor Haslizawati binti Abu Bakar on her degree thesis entitled "Chemical constituents, Bioactivity and HPLC Profiling of Microwave-Assisted and Normal Extraction of *Murraya koenigii*" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Degree of Master of Sciences.
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DECLARATION

I hereby declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

_____

NOOR HASLIZAWATI ABU BAKAR

Date: 17 December 2009

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4.73 HPLC chromatograms of methanol extracts from *Murraya koenigii* stem barks obtained from normal extraction (NE) and microwave-assisted extraction (MAE). Isolated compounds used were mahanimbine (1), girinimbine (2), and murrayanine (3), each concentration was 1 mg/mL. 213

4.74 HPLC chromatograms of hexane extracts from *Murraya koenigii* roots obtained from normal extraction (NE) and microwave-assisted extraction (MAE). Isolated compounds used were mahanimbine (1), girinimbine (2), murrayanine (3) and murayafoline-A (4), each concentration was 1 mg/mL. 215

4.75 HPLC chromatograms of chloroform extracts from *Murraya koenigii* roots obtained from normal extraction (NE) and microwave-assisted extraction (MAE). Isolated compounds used were mahanimbine (1), girinimbine (2), murrayanine (3) and murayafoline-A (4), each concentration was 1 mg/mL. 216

4.76 HPLC chromatograms of methanol extracts from *Murraya koenigii* roots obtained from normal extraction (NE) and microwave-assisted extraction (MAE). Isolated compounds used were mahanimbine (1), girinimbine (2),murrayanine (3) and murayafoline-A (4), each concentration was 1 mg/mL. 217

LIST OF ABBREVIATIONS

α	Alpha
β	Beta
δ	Chemical shift in ppm
^{13}C	Carbon-13
$CHCL_3$	Chloroform
ºC	Degree in Celcius
$CDCl_3$	Deutrated chloroform
COSY	Correlated spectroscopy
cm	Centimeter
J	Coupling constant in Hertz
d	Doublet
DEPT	Distortionless Enhancement by Polarisation Transfer
DMSO	Dimethylsulfoxide
EIMS	Electron Impact-MASS spectroscopy
EA	Ethyl Acetate
G	Gram
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectroscopy
^{1}H	Proton
HMBC	Heteronuclear Mutiple Bond Connectivity
HMQC	Heteronuclear Multiple Quantum Correlation
HPLC	High Performance Liquid Chromatogaphy
Hz	Hertz
OH	Hydroxy
IC	Inhibition Concentration
IR	Infrared
LC	Lethal concentration
m/z	Mass per charge
MS	Mass spectroscopy
MeOH	Methanol

OMe	Methoxy
Me	Methyl
m.p.	Melting point
mL	Milliliter
mm	Milimeter
µg	Microgram
µL	Microliter
MAE	Microwave-assisted extraction
mg	Milligram
M^+	Molecular ion
m	Multiplet
nm	Nanometer
NMR	Nuclear Magnetic Resonance
ppm	Part per million
KBr	Potassium bromide
s	Singlet
t	Triplet
TLC	Thin layer chromatography
UV	Ultraviolet
WHO	World Health Organization